S.7.3 DYNAMIC COLLAGEN V REMODELING IS RELATED TO SKIN THICKENING IN SSc

P. Martin[1], **W. R. Teodoro**[1], A. P. Velosa[1], S. Carrasco[1], J. de Morais[1], R. B. Christmann[2], E. R. Parras[3], V. L. Capelozzi[3] and N. H. Yoshinari[1]
[1]*Disciplina de Reumatologia da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil,* [2]*Boston University, Boston, MA, USA and* [3]*Departamento de Patologia da Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil.*

Background. Normal physiological properties of skin, one of the primary organs affected in SSc, depends on collagen Types I (COL I), III (COLIII) and V (COLV) assembly forming heterotypic fibres. COLV regulates fibril diameter and loss of this function could result in tissue fibrosis. In this way, our aim was to evaluate the histological and molecular profiles of COLI, COLIII and COLV in SSc skin and its correlation with skin thickening and disease activity.
Methods. Skin biopsies of 18 patients (5 at early and 13 at late disease stage) and 10 healthy controls were studied. Assessment of skin thickening was performed using the modified Rodnan skin score (MRSS) and disease activity was calculated by Valentini Disease Activity Index. Quantification of COLI, COLIII and COLV was evaluated by histomorphometry in dermis and quantitative RT–PCR in dermal fibroblast culture.
Results. A higher expression of abnormal COLV was observed in dermis of patients with early disease when compared with control group and late disease. The COLIII content was also higher in early SSc when compared with healthy controls and late SSc. On the other hand, the amount of COLI was higher in late disease when compared with control and early SSc. A positive correlation between COLV and MRSS ($r = 0.42$, $P = 0.04$) as well as disease activity ($r = 0.45$, $P = 0.03$) was observed, but there was no correlation between COLI and COLIII expression and these parameters. COLV α-1 and COLV α-2, as well as COLI α-1 and COLIII α-1 mRNA expression were higher in SSc when compared with control group.
Conclusion. We found increased COLIII and COLV deposition in early SSc and increased COLI expression in late SSc indicating that collagen remodelling in SSc is a dynamic process. The fact that abnormal COLV expression decreases in later disease stages could explain why skin thickening sometimes improves spontaneously with time. Besides, COLV is correlated to MRSS and disease activity. These findings include COLV as an important regulator of cutaneous thickness in SSc and may add this protein as a new target for future treatments.

S.7.4 TENDON INVOLVEMENT IN PATIENTS WITH SSc

G. Cuomo[1], M. Zappia[2], M. Iudici[1], G. Abignao[1] and G. Valentini[1]
[1]*Rheumatology Unit and* [2]*Radiology Unit, Second University of Naples, Naples, Italy.*

Objective. To investigate the ultrasonographic features of tendons and surrounding structures in patients with SSc.
Methods. Fifty-five SSc patients (27 with dcSSc, 28 with lcSSc) and 30 healthy control subjects were recruited. Tendon involvement was investigated by clinical examination and ultrasonography (with a General Electric Logiq 5 PRO using a 7–12 MHz linear array transducer) at MCP areas (extensor and flexor tendons), wrists (extensor and flexor tendons), knees (patellar), ankles (anterior, posterior, medial and lateral).
Results. Clinical examination revealed tenosynovitis in 11 SSc patients (8 lcSSc and 3 dcSSc) and no control subject. Tendon friction rubs (TFRs) were detected in 12 dcSSc patients. Two dcSSc patients presented both findings at the same tendons. US revealed tenosynovitis in 21 patients (including the 11 with the respective clinical finding). Tendons where TFRs had been identified at clinical examination presented tenosynovitis in 10% of the cases, all of whom presented a significantly increased retinaculum thickness (Table 1).

TABLE 1. Thickness of the retinacula in each investigated site and in tendons presenting tendon friction rubs at physical examination

Subjects	Retinacula thickness, median (range), mm			Ankle anterior
	Wrist extensors	Wrist flexors	Patellar	
Healthy subjects (30)	0.8 (0.6–1.4)	0.8 (0.6–1.4)	0.8 (0.6–1.4)	0.8 (0.6–1.4)
Lc-SSc (28)	0.8 (0.6–1.2)	0.8 (0.6–1.2)	0.8 (0.6–1.2)	0.8 (0.6–1.4)
Dc-SSc TFRs$^-$ (15)	0.8 (0.6–1.4)	0.8 (0.6–1.2)	0.8 (0.6–1.2)	0.8 (0.6–1.2)
Dc-SSc TFRs$^+$ (12)	1.5 (1.1–2.3)	0.8 (0.6–1.4)	1.2	1.4 (1.1–2.0)
P	*	>0.05	>0.05	**

*$P = 0.001$, dcSSc TFRs$^+$ *vs* HS; $P = 0.002$ dcSSc TFRs$^+$ *vs* lcSSc; $P = 0.001$ dcSSc TFR$^+$ *vs* dcSSc TFRs$^-$; * *$P = 0.002$, dcSSc TFR^{s+} *vs* HS; $P = 0.003$ dcSSc TFRs$^+$*vs* lcSSc; $P = 0.003$ dcSSc TFRs$^+$ *vs* dcSSc TFRs$^-$.

Conclusion. US revealed a higher prevalence of tendon involvement with respect to clinical examination in patients with SSc. Increased retinaculum thickness might be the morphological basis of TFRs, particularly in tendons devoid of synovial sheaths.

SESSION 8

PATHOGENESIS–BIOMARKERS/ IMMUNOLOGY

S.8.1 AN IMMUNOCHIP-BASED INTERROGATION OF SCLERODERMA SUSCEPTIBILITY VARIANTS

J. Charlesworth[1], J. Stankovich[1], P. Lewis[1], J. Byron[2], W. Stevens[2], J. Sahhar[3], S. Proudman[4], J. Roddy[5], P. Nash[6], K. Tymms[7], M. Brown[8] and J. Zochling[1]
[1]*Menzies Research Institute Tasmania, Hobart,* [2]*Department of Rheumatology, St Vincent's Hospital,* [3]*Department of Rheumatology, Monash Medical Centre, Melbourne,* [4]*Rheumatology Unit, Royal Adelaide Hospital, Adelaide,* [5]*Department of Rheumatology, Royal Perth Hospital, Perth,* [6]*Department of Medicine, University of Queensland, Maroochydore, Australia,* [7]*Canberra Rheumatology, Canberra and* [8]*Diamantina Institute, University of Queensland, Brisbane, Australia.*

Introduction. Understanding the genetic architecture of scleroderma (SSc) susceptibility is vital both in gene discovery and in determining the influence of previously identified susceptibility variants. It is particularly important in understanding disease mechanism in a disease with few therapies and great morbidity and mortality.
Methods. We selected 557 cases from the Australian Scleroderma Cohort Study (ASCS), for genotyping with the Immunochip, a custom Illumina Infinium genotyping array containing 196 524 rare and common variants shown to be important in a wide variety of autoimmune disorders. A total of 4537 controls were taken from the 1958 British Birth cohort. Genotype data were analysed with PLINK. Samples and SNPs with low call rates were excluded, as were SNPs in Hardy–Weinberg disequilibrium or with less than two occurrences of the minor allele. Eigenstrat was used to analyse population structure. The final data set consisted of 505 cases, 4491 controls and 146 867 SNPs. Allelic association analyses were conducted using Fisher's exact test. Genotype clusters were manually examined for all associations of $P < 10^{-5}$ since calling is difficult for some rare variants.
Results. Significant and suggestive associations were detected at seven loci. Several of these have been previously implicated in scleroderma susceptibility (HLA-DRB1 and STAT4) and several are novel associations, including SNPs near PXK ($P = 4.4 \times 10^{-6}$) and CFDP1($P = 2.6 \times 10^{-6}$).
The strongest associations were with SNPs in the Class II region of the MHC. One of the most strongly associated SNPs [rs4639334; $P = 1.6 \times 10^{-8}$; odds ratio (OR) = 1.8] is in linkage disequilibrium ($r^2 = 0.46$) with the Class II allele HLA-DRB1*11:01. This allele has been associated with SSc. Another strongly associated SNP is rs2857130 ($P = 1.6 \times 10^{-8}$; OR = 0.67), which lies in the promoter region of HLA-DRB1, but is not in LD with any classical MHC alleles. Outside the MHC, there were six regions of association with $P < 10^{-5}$,including the confirmed SSc locus at STAT4. Several SNPs implicate a locus at PXK, which has been previously associated with SLE but not with SSc. The remaining associations are novel for both SSc and SLE and require replication. Of particular interest is a rare variant located within a non-coding RNA on chromosome 6q21 which was ~20 times more frequent in cases than controls. We are currently dissecting the potential biological implications of this locus.

Conclusions. This pilot study has confirmed previously reported SSc associations, revealed further genetic overlap between SSc and SLE, and identified putative novel SSc susceptibility loci including a rare allele with major effect size.

S.8.2 LOSS OF PTEN, VIA A CCN2-DEPENDENT MECHANISM—RESULTS IN A SCLERODERMA-LIKE PHENOTYPE *IN VIVO*

A. Leask[1], S. Parapuram[1], X. Shiwen[2], C. Denton[2], D. Abraham[2] and S. Liu[1]
[1]*University of Western Ontario, London, Canada and* [2]*University College London, London, UK.*

Fibrosis represents a common pathway leading to organ failure and death in many diseases, including scleroderma, and has no effective therapy. Dysregulated repair and excessive tissue scarring provides a unifying mechanism for pathological fibrosis. The protein phosphatase and tensin homologue (PTEN) dephosphorylates proteins that promote tissue repair and thus could be a key fibrogenic mediator. PTEN expression is reduced in skin fibroblasts from patients with the fibrotic autoimmune disease diffuse SSc (dSSc) ($n = 6$, $P < 0.05$). To evaluate whether this deficiency could be sufficient for fibrogenesis *in vivo*, we used a conditional knockout strategy to deleted PTEN in adult mouse fibroblasts. Compared with littermate control mice, loss of PTEN resulted in a 1.5-fold increase in dermal thickness due to excess deposition of collagen and the presence of myofibroblasts ($n = 6$, $P < 0.05$). Moreover, lung fibrosis was also observed, including an increase in collagen production and the presence of myofibroblasts ($n = 6$, $P < 0.05$). PTEN-deleted skin and lung fibroblasts showed increased expression of connective tissue growth factor (CTGF/CCN2). Overexpression of PTEN reduced the overexpression of Type I collagen and CCN2 by dSSc fibroblasts ($n = 3$, $P < 0.05$). Double CCN2/PTEN mice did not get fibrosis; i.e. the absence of CCN2 rescued the effects of loss of PTEN in terms of collagen and myofibroblast production ($n = 6$, $P < 0.05$). Loss of PTEN resulted in increased expression of the proliferative marker PCNA; the loss of CCN2 did not resuce this phenotype ($n = 6$, $P < 0.05$). Thus, PTEN appears to be a potential *in vivo* master regulator of fibroproliferative conditions. CCN2 appears to mediate the fibrotic (collagen, myofibroblast) but not proliferative (PCNA) effect of loss of PTEN. Thus, PTEN agonists may represent therapies for fibroproliferative conditions, whereas CCN2 inhibition may specifically affect the fibrotic component of these diseases.

S.8.3 A MICRORNA SIGNATURE OF SSc

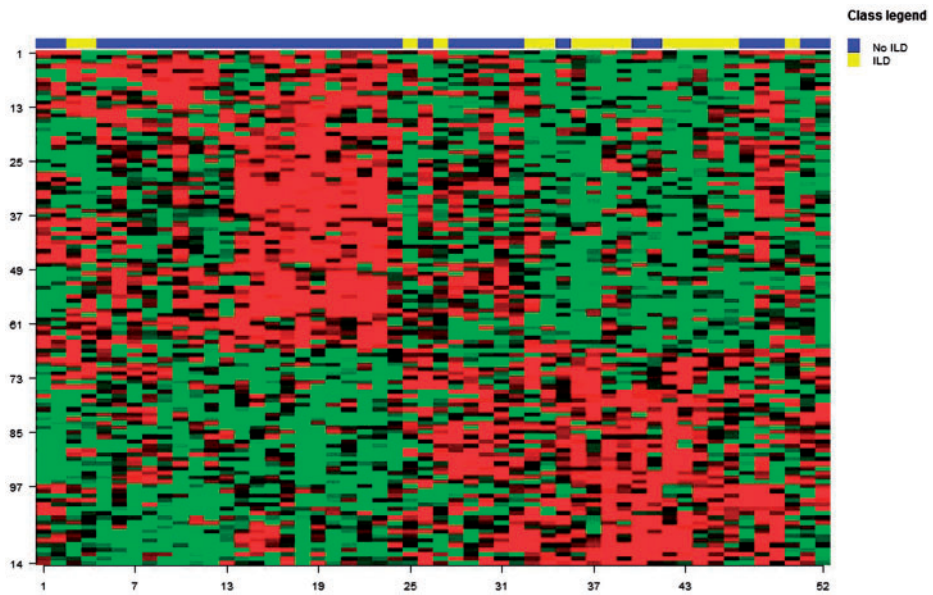
S. Vettori[1], M. Brock[1], N. Iwamoto[1], B. Maurer[1], A. Jungel[1], R. E. Gay[1], M. Calcagni[2], G. Valentini[3], J. H. Distler[4], S. Gay[1] and O. Distler[1]
[1]*Center of Experimental Rheumatology,* [2]*Division of Plastic Surgery and Hand Surgery, Zurich University Hospital, Zurich, Switzerland,* [3]*Rheumatology Unit, Second University of Naples, Naples, Italy and* [4]*Department of Internal Medicine 3, University of Erlangen-Nuremberg, Erlangen, Germany.*

Background. MicroRNAs (miRs) regulate many pathophysiological processes. Our aim was to investigate miR expression and function in SSc.
Materials and methods. A total of 377 miRs were profiled on three pooled SSc and healthy control (HC) fibroblasts by low-density array. Differentially expressed miRs were further investigated in 12 individual SSc and 6 HC fibroblasts by real-time PCR. MiR-145 expression was also investigated in 12 individual and 6 HC dermis samples. miR-145 targets were analysed under pre/anti-miR transfection in SSc and HC fibroblasts by real-time PCR and western blot.
Results. Twenty-six miRs were down-regulated and five up-regulated in pooled SSc fibroblasts. Basal down-regulation of miR-17-5p, miR-20a, miR-21, miR-24, miR-29a, miR-29b, miR-29c, miR-99a, miR-145, miR-186 and miR-193b, as well as miR-155 up-regulation (all $P < 0.05$) was confirmed in individual SSc fibroblasts. MiRs were regulated by multiple factors in SSc fibroblasts: hypoxia exposure induced miR-210 up-regulation ($P < 0.05$), while pro-fibrotic cytokines (TGF-β, PDGF-B and IL-4) further reduced miR-29a/b/c levels ($P < 0.05$). MiR-145 expression was modulated by epigenetic modifications, as inhibition of DNA methyltransferases by 5aza-C increased miR-145 levels by 25% in SSc fibroblasts. Both miR-145 and its primary transcript pri-miR-145 were also down-regulated in SSc dermis ($P < 0.001$) suggesting transcriptional regulation. MiR-145 was further investigated for effects on pro-fibrotic pathways: the miR-145 targets TGFBR2, SMAD3 and CTGF were decreased between 53% and 66% at both mRNA and protein level after transfection with pre-miR-145. Collagen Types I and III were similarly reduced. A reporter gene assay with the 3′-UTR of TGFBR2 showed reduction of the luciferase gene activity under miR-145 treatment by 34% ($P < 0.05$) indicating direct regulation of TGFBR2 by miR-145.
Conclusions. Our data show a profoundly impaired miR profile in SSc, which is regulated by multiple factors including epigenetic modifications. By up-regulating multiple pro-fibrotic factors on the post-transcriptional level, dysregulated miRs appear important molecular players in fibrogenesis. In particular, down-regulation of miR-145 showed strong pro-fibrotic effects in SSc by inducing multiple components of the TGF-β signalling pathway.

S.8.4 GLOBAL GENE EXPRESSION PROFILING IN SKIN IDENTIFIES TRANSCRIPTS CORRELATING WITH SEVERITY OF INTERSTITIAL LUNG DISEASE IN SSc

S. Assassi[1], M. Mayes[1], X. Liu[1], B. Harper[2], E. Gonzalez[2], H. Draeger[3], X. Zhou[1], D. Khanna[4], D. Furst[5] and F. Tan[1]
[1]*University of Texas Health Science Center at Houston, Houston, USA,* [2]*University of Texas Medical Branch, Galveston, USA,*

FIG. 1



[3]*University of Texas Health Science Center at San Antonio, San Antonio, USA,* [4]*University of Michigan, Ann Arbor, USA and* [5]*University of California Los Angeles, Los Angeles, USA.*

Objective. We conducted global gene expression profiling to identify transcripts in skin biopsy samples of patients with SSc that correlate with severity of interstitial lung disease (ILD).
Methods. Skin biopsy samples were obtained from 52 patients enrolled in the Genetic *vs* Environment in Scleroderma Outcome Study (GENISOS). RNA was extracted, amplified and hybridized on Illumina HumanHT-12 arrays. Skin transcript levels correlating with concomitantly obtained per cent predicted forced vital capacity (FVC; a validated surrogate with severity of ILD) were identified by quantitative trait analysis.
The plasma level of a candidate gene (*CCL2*) identified by the skin gene expression analysis was examined in all patients enrolled in the GENISOS cohort ($n = 266$) to determine its correlation with FVC.
Results. Fifty-two patients (diffuse cutaneous involvement = 59%, disease duration = 7.73 years, presence of ILD = 31%) were examined by global gene expression profiling. There were 109 skin transcripts that significantly correlated with FVC levels. We performed unsupervised hierarchical clustering of SSc samples using this gene list (114 transcripts). As shown in Fig. 1, 87.5% (14/16) patients with an FVC < 70% clustered together (colour-coded yellow in Fig. 1) while the majority of patients (21/36 = 58%) with FVC > 70% clustered in a separate group (colour-coded purple in Fig. 2). Next, we modelled the significantly over-expressed genes in patients with more severe ILD (FVC < 70%) in the Ingenuity Pathways Knowledge Base (v9.0). The genes belonging to pathways involved in chemotaxis and adhesion of inflammatory cells were significantly over-represented ($P = 2.1 \times 10^{-7}$). These genes included *CCL2*, *CCL4*, *CCL5*, *CXCR4*, *SELE* (*CD62E*) and *SELP* (*CD62P*).
CCL2 (MCP-1), a transcript closely correlating with FVC ($r = -0.37$; $P = 0.007$) was selected for our confirmatory plasma studies in the GENISOS cohort. CCL2 levels were determined in baseline plasma samples in the GENISOS cohort ($n = 266$ disease duration <5 years). The plasma CCL2 levels inversely correlated with the concomitantly obtained FVC in the univariable analysis ($r = -0.18$, $P = 0.008$). This relationship remained significant ($P = 0.013$) after adjustment for potential confounding effect of gender, age, ethnicity, smoking status, disease duration, disease type or treatment with immunosuppressive agents. This indicates that higher CCL2 levels correlate with more severe ILD in SSc.
Conclusion. Skin gene expression levels belonging to pathways involved in chemotaxis and adhesion of inflammatory cells correlate with severity of lung disease in SSc. This finding can lead to identification of novel therapeutic targets and biomarkers for more focused and effective treatment of SSc-ILD.

SESSION 9

IMAGING

S.9.1 LUNG ULTRASOUND FOR THE SCREENING OF INTERSTITIAL LUNG DISEASE IN SSc

T. Barskova[1], **L. Gargani**[2], M. L. Conforti[1], S. Guiducci[1], C. Bruni[1], A. Moggi Pignone[3], E. Picano[2] and M. Matucci Cerinic[1]
[1]*Department of Biomedicine, Division of Rheumatology, Excellence Centre for Research, Transfer and High Education DENOthe, Florence,* [2]*Institute of Clinical Physiology, National Research Council, Pisa and* [3]*Department of Internal Medicine, University of Florence, Florence, Italy.*

Background. A high percentage of SSc patients develop interstitial lung disease (ILD) during the course of the disease. Promising data have recently shown that lung US (LUS) is able to detect ILD by the evaluation of B-lines (previously called US lung comets), the sonographic marker of pulmonary interstitial syndrome.
Objective. To evaluate whether LUS could be employed for an early screening of ILD in patients with a very early diagnosis of SSc.
Methods. Sixty-eight consecutive SSc patients [65 females, mean age 51 (13) years] who underwent a clinically driven chest high-resolution CT (HRCT) were evaluated by LUS for detection of B-lines. Among them, 24 patients fulfilled the criteria for a Very Early Diagnosis of SSc (VEDOSS).
Results. ILD was present at HRCT in 69% of the total population and in 65% of the VEDOSS population. A significant positive linear correlation was found between B-line numbers and the presence of ILD at HRCT ($r = 0.55$; $P < 0.001$) in all patients. When considering only the VEDOSS population, the concordance rate between the two examinations was 88%, with a sensitivity of 96%, a negative predictive value of 88%, specificity of 50% and positive predictive value of 78%.
Conclusions. ILD appears very early in SSc patients. Presence of B-lines at LUS examination correlates with ILD at HRCT. LUS is very sensitive to detect early ILD even in patients with very early diagnosis of SSc. The use of LUS as a screening tool for ILD seems feasible to guide further investigation with HRCT.

S.9.2 B-LINES AS A LONG-TERM PROGNOSTIC DETERMINANT IN SSc

L. Gargani[1], S. Guiducci[2], M. Doveri[3], G. Agoston[4], A. Moreo[5], F. Musca[5], E. Bruschi[6], O. Epis[6], S. Bellando Randone[2], C. Bruni[2], L. Bazzichi[3], S. Bombardieri[3], A. Varga[4], R. Sicari[1], M. Matucci Cerinic[2] and E. Picano[1]
[1]*Institute of Clinical Physiology, National Research Council, Pisa,* [2]*Department of Biomedicine, Division of Rheumatology, Excellence Centre for Research, Transfer and High Education DENOthe, Florence,* [3]*Department of Internal Medicine, Rheumatology and Immunoallergology Units, Pisa, Italy,* [4]*2nd Dept of Internal Medicine and Cardiology Center, Szeged, Hungary,* [5]*Cardiology Department and* [6]*Rheumatology Department, Niguarda Ca' Granda Hospital, Milan, Italy.*

Background. Pulmonary arterial hypertension and pulmonary fibrosis are established prognostic determinants in patients with SSc. Pulmonary hypertension [by pulmonary artery systolic pressure (PASP)] and fibrosis (by B-lines, also called US lung comets) can be measured in a one-stop shop with cardiac and lung US.
Purpose. To assess the prognostic value of B-lines and PASP in SSc.
Methods. A total of 68 SSc patients [age 56 (14) years, 63 females] admitted to the Rheumatology Division of the University of Pisa were evaluated with a comprehensive 2D and Doppler echocardiography, and lung US with B-lines assessment. B-lines score of a patient was obtained by summing the number of B-lines found on anterior and posterior chest. PASP was calculated from the maximal velocity of tricuspid regurgitation flow.
Results. During follow-up [median (interquartile range) 25 (21–31) months], 23 events occurred: 4 deaths, 3 admissions for worsening skin involvement and 16 for worsening dyspnoea. An ROC analysis

FIG. 1